DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

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Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH

and

COLLABORATING INVESTIGATORS

ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)
Vironostika® HIV-1 Antigen Microelisa System
Organon Teknika

I. PRINCIPLE

The Human Immunodeficiency Virus Type 1 (HIV-1) is recognized as the etiologic agent of acquired immunodeficiency syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected body fluids or tissues, and from mother to fetus or child during perinatal period. After exposure to the virus, HIV-1 infection is characterized by an early period of antigenemia in which HIV-1 antigens (Ag) are detectable in blood. In most individuals the antigen level becomes undetectable for a period of time; late in disease, increasing failure of the immune system and increasing levels of virus may again result in detectable levels of antigen. One of the viral components in blood during antigenemia is the core protein, p24, the major internal structural protein of HIV-1.

The Vironostika® HIV-1 p24 Antigen Microelisa System is an enzyme immunoassay (EIA, or Enzyme-linked Immunoabsorbant Assay, ELISA) developed for detection and quantitation of the HIV-1 p24 core protein. The Vironostika HIV-1 assay uses a murine monoclonal antibody to HIV-1 p24 antigen coated onto microtiter strip wells. A specimen of plasma, serum or tissue culture medium and lysis buffer are added to a coated well and incubated. If present, the viral antigens bind to the monoclonal antibody on the microtiter well. Subsequently, anti-HIV-1 (human) conjugate labeled with HRP is added. The labeled antibody binds to the solid phase antibody/antigen complexes previously formed. In a final step, a substrate reagent containing tetramethylbenzidine (TMB) and hydrogen peroxide is added which reacts with complexed peroxidase to form a blue color. The reaction is terminated by the addition of acid, and the absorbance is measured spectrophotometrically. The intensity of the color development is directly proportional to the amount of uncomplexed p24 antigen in the plasma, serum or tissue culture media. The quantity of free HIV-1 p24 antigen in a specimen is determined by comparing its absorbance with that of known HIV-1 p24 antigen standard curve.

II. SPECIMEN REQUIREMENTS

Serum, tissue culture supernatant or plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate or heparin may be used and should be tested as soon as possible following collection. If the situation limits the ability to test the sample quickly, the specimen can be held in refrigeration (2-4^{0}C) for a maximum of 7 days. If the period of time will be greater, the sample can be held at -20^{0}C or -85^{0}C for long term storage.

Remove the serum from the clot, or plasma from the red cells as soon as possible to avoid hemolysis.

Specimens containing particulate matter may give inconsistent results. Such specimens should be clarified by centrifugation prior to assay.

Avoid subjecting specimens to repeated freeze thaw cycles.

Bring all specimens to room temperature (15-30^{0}C) prior to assay.

III. REAGENTS

A. The Vironostika® HIV-1 Antigen Microelisa Assay, 192 (PN 59464) kits include the following reagents:.

1. HIV-1 p24 Antibody-coated Microelisa Strips. Store at 2-8^{0}C. Note manufacturer's outdate.

    a. Bring pouch containing HIV-1 p24 antibody coated microtiter strips to room temperature (15-30^{0}C) before opening to avoid condensation on the strips.

    b. The plate consists of 8 removable strips of 12 wells each. Any partial use of strips commits all 8 wells to the assay. Antibody coated strips may be used only once. When using a 96 well plate washer and fewer than 8 strips are needed, place uncoated strips in the remaining positions.

    c. Unused strips may be placed back into the pouch and sealed with the desiccant provided and stored at 2-8^{0}C for 60 days.

2. Anti-HIV-1 Conjugate (human)-Horseradish peroxidase-labeled anti-HIV-1. Store at 2-8^{0}C. Note manufacturer's outdate.

3. Disruption Buffer. Store at 2-8^{0}C. Note manufacturer's outdate.

4. TMB Solution. Store at 2-8^{0}C. Note manufacturer's outdate.

    a. Within 10 minutes of use prepare the TMB-Substrate Solution by adding 1 mL of TMB Substrate to 1 mL of Peroxidase Solution for each Microelisa Strip that will be used.

5. Peroxide Solution. Store at 2-8^{0}C. Note manufacturer's outdate.

6. Phosphate Buffer Concentrate. Store at 2-8^{0}C. Note manufacturer's outdate.

a. Prepare at least 50 mL of Phosphate Buffer working solution for each Microelisa Strip used. Note: Approximately 700 mL of working solution is required for a complete 96 well plate.

b. Dilute the Phosphate Buffer Concentration 1:25 with distilled water.

c. Discard any unused reagent at the end of the day.

7. Stop Solution (2N H_2SO_4). Store at 2-30^{0}C. Note manufacturer's outdate.

8. Negative Control - human serum nonreactive for HIV-1 antibody. Note manufacturer's outdate.

9. Positive Control - human HIV-1 antigen containing 160 pg/mL p24 core antigen (inactivated). Note manufacturer's outdate.

B. Reagents required but not provided:

1. 5% Hypochlorite solution (household bleach) diluted 1/100 or appropriate disinfectant.

2. Deionized or distilled water.

3. Standards and controls for the assay provided by the Virology Quality Assurance Laboratory (VQA):

a. VQA SQC (Serum Quality Control). A set of five concentrations. Store at -80^{0}C.

1) Just prior to set up, thaw 1 vial of each of the 5 concentrations.
2) Mix well and use.

IV. SUPPLIES AND EQUIPMENT

Lab coat
Gloves
Micropipet(s) capable of delivering 10 µL, 20 µL, 50 µL, 200 µL volumes
Multichannel pipette(s) capable of delivering 10 µL, 20 µL, 50 µL, 200 µL volumes
Disposable pipette tips suitable for the above pipettes
Disposable reagent reservoirs
Microelisa Strip Holder
Disposable vials
Serological pipettes
Incubator without CO_2 capable of maintaining 37^{0}C +/- 2^{0}C

Centrifuge
Timer capable of measuring times up to 60 minutes
Graduated cylinders and beakers
ELISA microtiter plate washer with waste trap and vacuum source
ELISA microtiter plate reader capable of measuring absorbance at 450 nm with reference at 630 nm

V. PROCEDURE

A. Plate Set-up

1. Bring all reagents and samples to room temperature.

2. Create an EIA template in the virology data-management software (see software manual).

3. Position the required number of microtiter strips in the strip holder reaction plate (12 wells per strip). If fewer than 8 strips are needed, use uncoated strip(s) in the remaining positions when using a 96 well plate washer.

4. Add 25 µL of Disruption Buffer to each test well of the coated microtiter plate.

5. Add 100 µL of each VQA SQC concentration and each specimen to the coated microtiter plate according to the template. Cover the plate using an adhesive plate cover.

6. Incubate at 37^0C for 1 hour $\pm$ 2 minutes.

7. Wash as follows: Aspirate the solution from the wells. Add 300 µL of Wash Buffer Working Dilution to each well. Allow wells to soak for 25-30 seconds. Aspirate the solution from the wells. Wash three (3) more times for a total of 4 washes. After the final wash step, grasp the plate firmly along the edges, invert plate over absorbent paper and tap the plate gently to remove any remaining liquid.

   Important: The time between the wash step and the next reagent must be less than five (5) minutes.

8. Add 100 µL of Anti-HIV-1 Conjugate to all testing wells (prepared during the last 10 minutes of incubation described above). Cover the plate using a new adhesive plate cover. Incubate at 37^0C $\pm$ 2 for 1 hour $\pm$ 2 minutes.

9. Wash as described above.

10. Add 100 µL of TMB Substrate to all testing wells. Incubate at room temperature (15^0C -30^0C) for 30 $\pm$ 2 minutes. Do not cover plate.

11. Add 100 µL of Stop Solution to all wells and mix by tapping.

    Important: Add Stop Solution to the wells in the same sequence and at the same rate of speed that the TMB Substrate was added.

12. Read absorbance at 450 nm (use 630 nm reference filter for dual wavelength instruments) within 15 minutes of adding Stop Solution to the wells. Blank the reader on air prior to reading.

VI. CALCULATIONS

The HIV-1 p24 antigen concentrations may be generated from a virology data-management software program developed for the Division of AIDS (DAIDS) to ensure data integrity of both QA and test specimens. A weighted linear least squares method using the VQA SQC concentrations is used to estimate HIV-1 p24 antigen concentration.

VII. QUALITY CONTROL

The absorbances obtained form the spectrophotometer may be transferred into the virology data-management software program. The software program incorporates two QC check programs, Cum Sum and Levy Jennings. These two programs review the absorbance of the VQA SQC and compare them to established standard deviation ranges. These ranges are determined by the testing laboratory and is reflective of values unique to each laboratory. The software will flag values that fall outside of the laboratory's standard deviation range. The technician must determine the significance of the out of range QC and resolve the situation.

VIII. PROCEDURAL NOTES

All pipetting steps should be performed with the utmost care and accuracy. Cross contamination between reagents and specimens will invalidate results. Use micropipettes for quantitative delivery of samples and reagents.

Use only reagents from the same kit lot.

To avoid contamination, do not touch the top of the Microelisa tips or the edge of the wells with fingers or pipette tips.

The aspiration/wash system should be flushed with copious amounts of water upon completion of the final wash of the assay.

Incomplete washing will adversely affect the test outcome. Phosphate Buffer must be at room temperature before use.

IX. REFERENCES

Vironostika® HIV-1 Antigen Microelisa System Assay package insert and all references within.